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To: Dr. D. Mills
Director of Research

NONLINEAR MODELLING OF PRAWN GROWTH - RATION CURVES

by

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The Biology Section of the Chemical Research Department conducted an experiment to estimate the relationship between prawn growth and size of food ration. The Statistics Unit was asked to analyse the experimental data. A nonlinear equation was used to model the growth - ration relationship. Several important growth parameters such as optimum ration size, minimum ration size yielding maximum growth and the rate of growth in terms of ration size were derived from this curve.

1. INTRODUCTION

The Biology Section of the Chemical Research Department has been conducting a series of experiments to study methods of raising prawns (large freshwater shrimp) efficiently and economically. The purpose is to make commercial use of the warm water available near Ontario Hydro's generating stations, in which prawns could adapt well. Prawns are of particular interest because they are a premium priced food product as well as have a rapid growth rate and are easy to raise in captivity. One of these experiments was conducted to study the effect of ration size on the growth rate of prawns.

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This report contains the statistical analyses of the growth - ration studies. The objective is to model the growth - ration curve from which important parameters can be estimated. One such parameter is the smallest size of food ration that yields the maximum growth of prawn. In addition, the curve describing the rate of growth per ration size versus ration size, called the conversion efficiency curve, is estimated from the growth - ration curve. Recommendations are made on how the experimental design can be improved.

2. THE DATA

Three weights of prawns were used in the study since the effect of ration size on growth depends on the initial size of the prawn. The selected weight classes of animals were 2, 5, and 15 grams. Prawns of each weight class were given six different sizes of food ration for six weeks; each prawn was on the same diet for the duration of the experiment.

The three weight classes of prawns were studied using separate experiments. Each experiment was set up using a Latin Square design, a statistically efficient method for distributing treatments to experimental units/1/. At the beginning of an experiment, prawns were equally divided among six tanks. There were twenty prawns per tank for each of the weight classes of 2 and 5 grams. Ten prawns were allocated per tank for the weight class of 15 grams. All prawns in a particular tank were given the same food ration size.

To control for tank effects, the ration size treatments were rotated from tank to tank once a week so that by the end of the experiment, each treatment had been applied to each tank. This involved moving the prawns. However, the risk of mortality did not increase since the prawns had to be removed from the tank anyway once a week when the tank was cleaned.

For the duration of the experiment, each prawn was given a particular size of food ration, which was measured as percent body weight of the prawn. The six food ration sizes given to 2 gram animals were O, 1, 3, 5, 7, and 9 percent of body weight; the six food ration sizes given to 5 gram animals were O, 1, 2, 3, 5, and 7 percent of body weight; and the six food ration sizes given to 15 gram animals were O, 0.75, 1.5, 2.25, 3, and 4 percent of body weight.

At the end of the experiment, the percent weight gain per day was computed for each prawn. This value is the measure of growth used throughout the analyses.

3. GROWTH - RATION MODELLING

In this section, the growth - ration curve, growth parameters which are estimated from the curve, and the conversion efficiency curve are discussed.

3.1 The Growth - Ration Curve

Figures 1, 2, and 3 show the raw data for the 2, 5, and 15 gram animals respectively. These figures indicate that the appropriate curve for fitting the data must start at a negative percentage value for the ration size of O and increase gradually to a maximum positive growth rate at which the growth rate levels off, called the asymptote of the curve.

A nonlinear model was chosen which has the properties described above. Mathematically, the model is expressed as:

$$g = a + b \exp(c\ r) + e \qquad (1)$$

where a (a positive value), b (a negative value), and c (a negative value) are parameters of the model which are to be estimated, exp is the exponential function, e is the random error term, g is the percent weight gain per day, and r is the ration size measured as percent body weight. The assumptions are made that the random error terms are independently and normally distributed with equal variances. It is noted that no linear transformation of the model is available that would make the model linear in the parameters indicating that a nonlinear regression method is required for fitting the data.

The curve in equation (1) levels off when g approximates a. It can also be negative for a ration size of O if a + b is negative. The parameter c, if negative, controls the rate at which the curve increases.

3.2 Growth Parameters

Several important growth parameters can be estimated from the growth - ration curve and are listed below.

- Optimum ration size (Ropt) - The point at which the ratio of growth to ration size is at a maximum. This is the most economical ration size.
- Optimum growth (Gopt) - Growth calculated at Ropt.
- Maximum growth rate (Gmax) - The maximum limit for growth that can be obtained at any ration size. Intuitively, growth rate increases asymptotically to this value as ration size increases.
- Maximum ration size (Rmax) - The minimum ration size which gives Gmax.
- Maintenance ration size (Rmaint) - The ration size at which there is O percent growth per day.
- Starvation growth rate (Gstarve) - The growth rate when no ration is given. This value is usually negative reflecting weight loss.

These parameters can be estimated from the growth - ration curve as described below.

- Gstarve is equal to a + b, since exp(c r) equals one when ration equals zero.
- Ropt is calculated by finding the point on the curve where the tangent goes through the point at which ration size is zero and percent weight gain per day is zero.
- Rmaint is calculated by solving equation (1) for ration and setting gain to O.

- Gmax is equal to a, since b exp(c r) approaches zero when ration approaches infinity.

- Since there is theoretically no Rmax for which the corresponding growth is Gmax, it is suggested that a lower bound on the asymptote be used above which Gmax is negligibly greater. For example, if Gmax is 0.8% weight gain per day, 0.75% will do as well. Rmax corresponding to 0.75% can then be calculated.

3.3 Conversion Efficiency Curve

The conversion efficiency curves for the three sizes of animals were computed directly from the estimated curves by dividing growth by ration size on both sides of the estimate of equation (1) and multiplying by 100 to obtain percent conversion efficiency. This curve usually has the shape of a dome which tapers off as ration sizes increases. The maximum point on the dome is at the optimum ration size, Ropt.

4. RESULTS

Before modelling the growth curves, the tank rotations were tested for significant differences. If a significant tank effect was obtained, then the random error terms in equation (1) would not be independent and the modelling would therefore be invalid. Computations were done using the analysis of variance procedure (PROC ANOVA) of the statistics packaged program, the Statistical Analysis System (SAS). A three-way analysis of variance was performed on factors week, ration size, and tank omitting all interaction terms. The results of the three tests are given in Table II. For each weight class of prawn, the tank effect was not significant at the 5% level of significance. Thus, the modelling of the growth - ration curves can proceed without violating the criterionthat the errors are independent.

4.1 The Growth - Ration Curve

The data in each weight class were tested using one-way analysis of variance to determine if there are significant differences between ration sizes. Significant differences were found for all three weight classes at the 1% level of significance ($F_{5,105} = 39.95$, $F_{5,112} = 55.35$, $F_{5,54} = 9.85$ for size classes 2, 5, and 15, respectively). Thus a model with more than a constant term is appropriate for describing the data.

The model was fit for each weight class of prawn using the statistics packaged program, BMDP/PC. The nonlinear regression procedure 3R was used which computes least squares estimates of the parameters by a Gauss-Newton algorithm. The fitted values for a, b, and c are shown in Table I with their asymptotic standard deviations. The standard deviations are only asymptotic since the nonlinear regression analysis does not produce exact estimates. A test for lack-of-fit for each size class showed no significant lack-of-fit at the 5% level of significance ($F_{3,105} = 1.03$, $F_{3,112} = 1.11$, $F_{3,54} = 0.983$ for 2, 5, and 15 gram animals, respectively). The residuals were normally distributed at the 5% level of significance. Figures 4, 5, and 6 show normal probability plots of the residuals for each weight class.

An R-square value provides a measure of how good the model fits the data and lies between O and 1, with 1 indicating a perfect fit. The models do not appear to fit very well since the R-square values for the 2, 5, and 15 gram experiments are O.64, O.67 and O.46, respectively. This is due to the large vertical scatter of the data (see Figures 1, 2, and 3) which leads to small R-square values. Maximum R-square values are obtained if the means are fit to each ration size. They are O.66, O.71, and O.48 for 2, 5, and 15 gram experiments, respectively. Thus, in reality, the R-square values obtained using the model are almost as high as those obtained from the best fit.

The standard errors for the parameters are only approximate since the nonlinear analysis does not yield the best estimates of the standard errors. However, they can be used to perform simple tests of significance using the asymptotic normality property. For each size class of prawn, a, b, and c are significantly different from zero at the 5% level of significance. The a parameters are different for size classes 2 and 15 grams and 5 and 15 but not for 2 and 5. The b parameters are equal for all size classes as are the c parameters. It is concluded that the 15 gram curve is significantly different from the other two.

Figures 1, 2, and 3 show the fitted curve overlaid on the original data for each size class. For 2 and 5 gram animals, the curve appears to overestimate the actual data at ration size 1% though it fits the other ration size levels well. The curve appears to be fitting very well for the 15 gram prawns. These observations may be due to a number of factors.

- The food ration sizes are not comparable between animals of weight class 15 grams and the other two weight classes. Animals of weight class 15 had sizes O, O.75, 1.5, and so on while weight classes 2 and 5 had O, 1, and so on.

- The curve may appear to be overestimating the data for weight classes 2 and 5 at the 1% ration size for reasons independent of food ration size. For example, the batch of prawns given the 1% ration size may have been subjected to different conditions than the other prawns even though strict control over environmental conditions was maintained.

- A better fit may have been obtained if there were more measurements taken for ration sizes less than 2% of body weight.

4.2 Growth Parameters

The growth parameters were estimated as described in Section 3.2 and the results given in Table I. The standard error for the asymptote, Gmax, is equivalent to that for the parameter a. In general, though, standard errors were not estimated for the growth parameters since the curves are nonlinear making the estimation procedure nonstandard. Bootstrapping, a simulation technique which does not require assumptions about the distribution of the parameters, provides one method of calculating standard errors/2/. To bootstrap, hundreds of random samples are taken from the existing data and the parameter calculated for each sample. The distribution for the parameter can then be empirically determined. This is beyond the scope of the project.

4.3 Conversion Efficiency Curve

Figures 7, 8, and 9 show the conversion efficiency curve for each size class of prawn. Each curve has the expected tapering dome-like shape.

5. CONCLUSIONS

- The nonlinear growth - ration model fits well except for the increasing part of the curve.
- The 2 and 5 gram growth - ration curves are not significantly different from each other and the 15 gram curve is significantly different from the other two. This would imply that policies for cultivating prawns of sizes 2 and 5 grams would be the same, but different for the 15 gram animals. However, since no tests of significance were developed for the growth parameters, aside from the model parameters, the extent of this difference is unclear.
- If derived directly from the growth - ration curve, the conversion efficiency curve had the expected dome-like shape.

6. RECOMMENDATIONS

- More ration size treatments should be given in the initial part of the curve to provide better fit for the increasing part of the curve.
- Standard errors of the optimal ration size, Ropt, should be calculated using bootstrap techniques.
- The lower bound for Gmax should be determined so that Rmax can be calculated.

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1. Cox, D.R., "Planning of Experiments". John Wiley & Sons, New York, 1958, P. 35 - 44.

2. Efran, B., and Tibshirani, R., "The Bootstrap Method for Assessing Statistical Accuracy". Technical Report No. 101, Division of Biostatistics, Stanford University, Stanford, California, March 1985.

TABLE I

Parameter Estimates and Standard Deviations from Growth - Ration Curve Modelling for Each Weight Class of Prawn

Note: Standard Errors are in Brackets

	Prawn Size		
Parameter	2 Gram	5 Gram	15 Gram
a	1.237 (0.086)	1.074 (0.060)	0.775 (0.085)
b	-1.567 (0.116)	-1.40 (0.092)	-1.085 (0.158)
c	-0.508 (0.109)	-0.762 (0.123)	-1.797 (0.747)
Ropt	1.672	1.214	0.590
Gopt	0.571	0.513	0.397
Gmax	1.237 (0.086)	1.074 (0.060)	0.775 (0.085)
Rmaint	0.479	0.354	0.188
Gstarve	-0.33	-0.33	-0.31

TABLE II

ANALYSIS OF LATIN SQUARE DESIGN FOR EACH WEIGHT CLASS OF PRAWN

Weight Class 2 Grams

Analysis of Variance Procedure

Dependent Variable: MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	0.60694354	0.04046290	5.30	0.0004
Error	20	0.15261734	0.00763087		
Corrected Total	35	0.75956088			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Week	5	0.31672414	0.06334483	8.30	0.0002
Ration	5	0.22972911	0.04594582	6.02	0.0015
TANK	5	0.06049029	0.01209806	1.59	0.2097

Weight Class 2 Grams

Dependent Variable: MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	2.62552854	0.17503524	4.15	0.0010
Error	20	0.84406021	0.04220301		
Corrected Total	35	3.46958875			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Week	5	1.66157503	0.33231501	7.87	0.0003
Ration	5	0.83271878	0.16654376	3.95	0.0119
TANK	5	0.13123473	0.02624695	0.62	0.6848

Weight Class 15 Grams

Dependent Variable: MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	17.38700000	1.15913333	6.24	0.0001
Error	20	3.71642222	0.18582111		
Corrected Total	35	21.10342222			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Week	5	5.5228556	1.1045711	5.94	0.0016
Ration	5	11.4461889	2.2892378	12.32	0.0001
TANK	5	0.4179556	0.0835911	0.45	0.8084

FIGURE 1

Weight Class 2 Grams Scatter Plot
of Growth Data with Estimated Growth Ration Curve

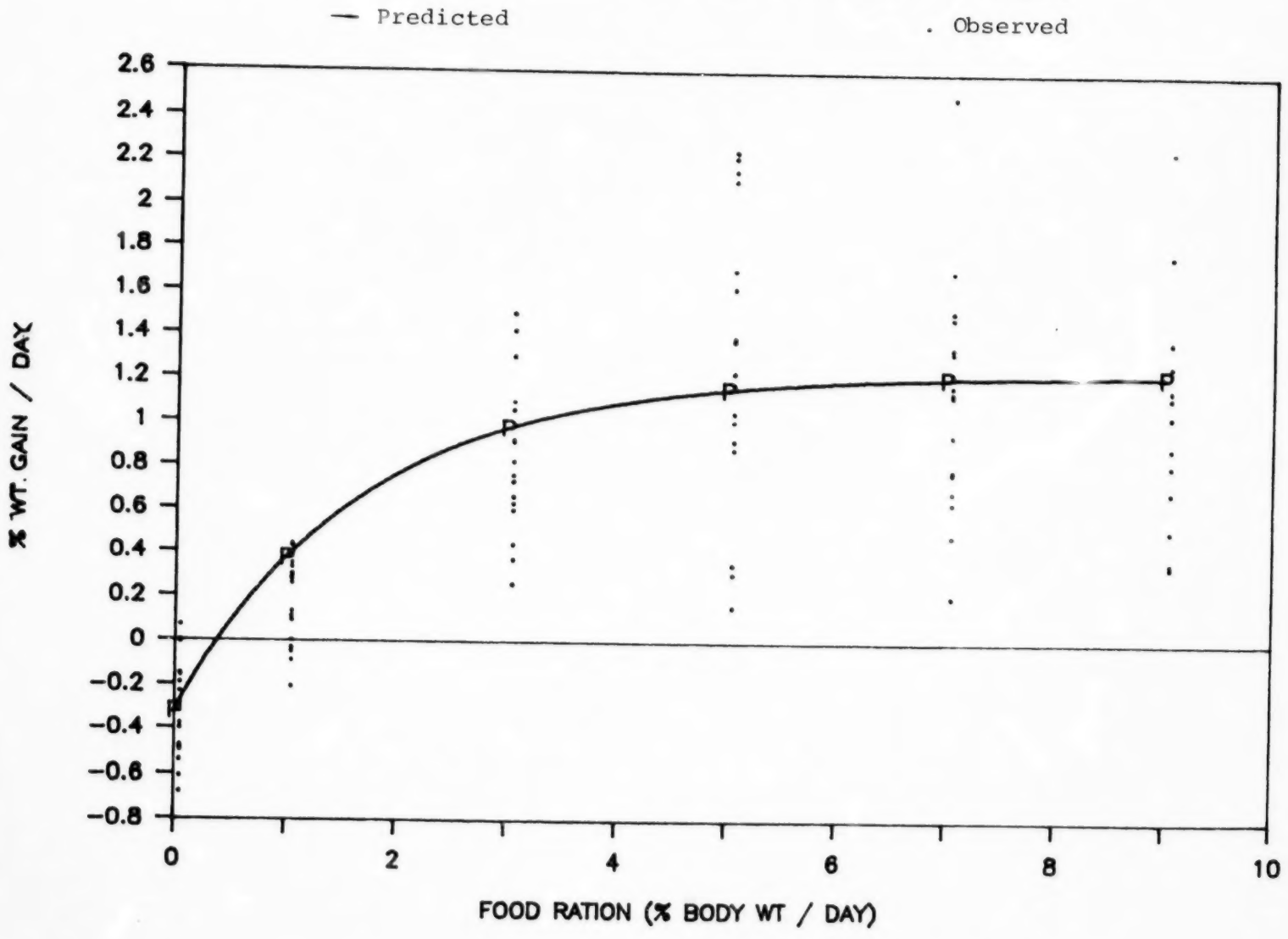


Figure 2

Weight Class 5 Grams Scatter Plot
of Raw Data with Estimated Growth Ration Curve

— Predicted . Observed

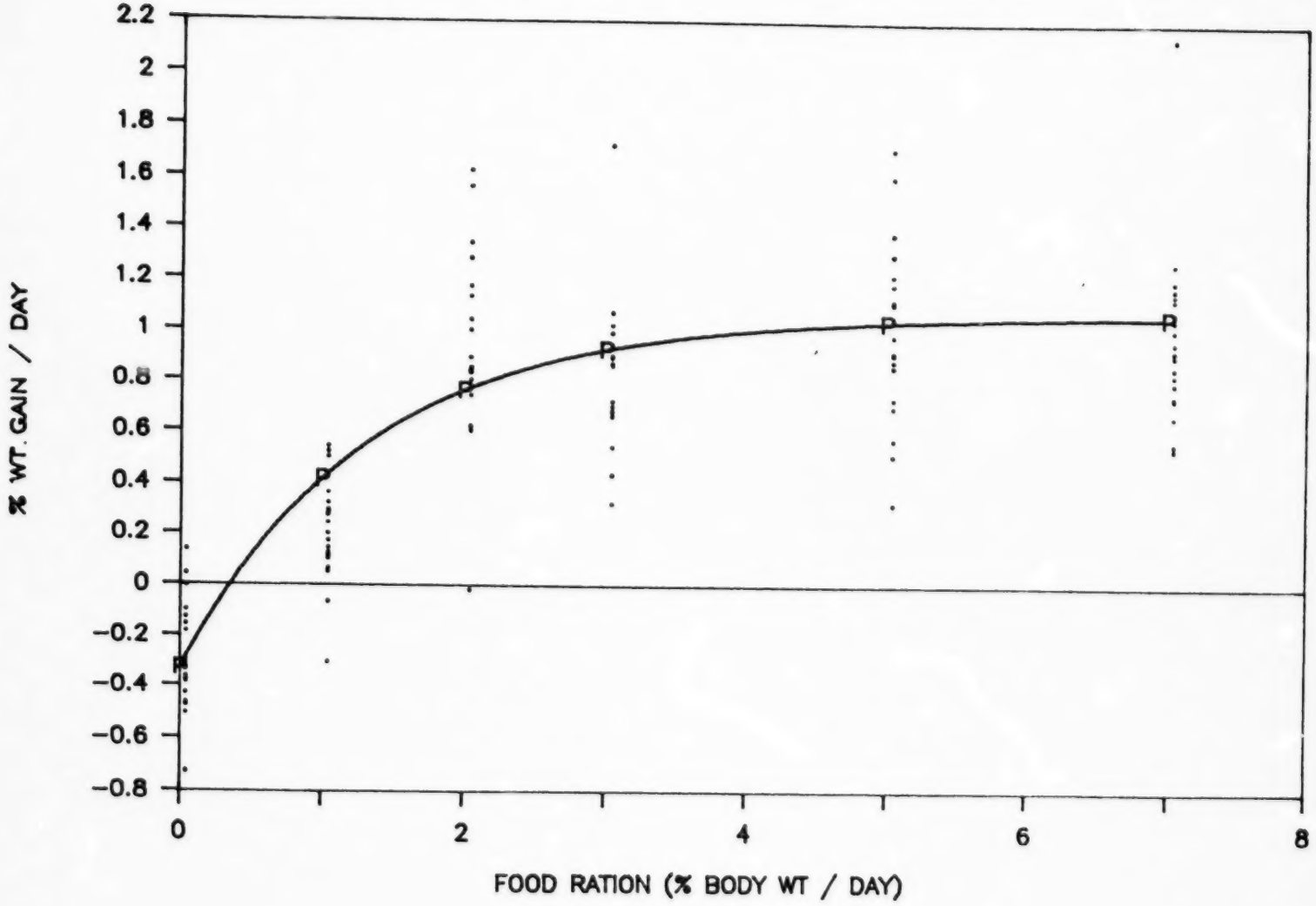


FIGURE 3

Weight Class 15 Grams Scatter Plot
of Raw Data with Estimated Growth Ration Curve

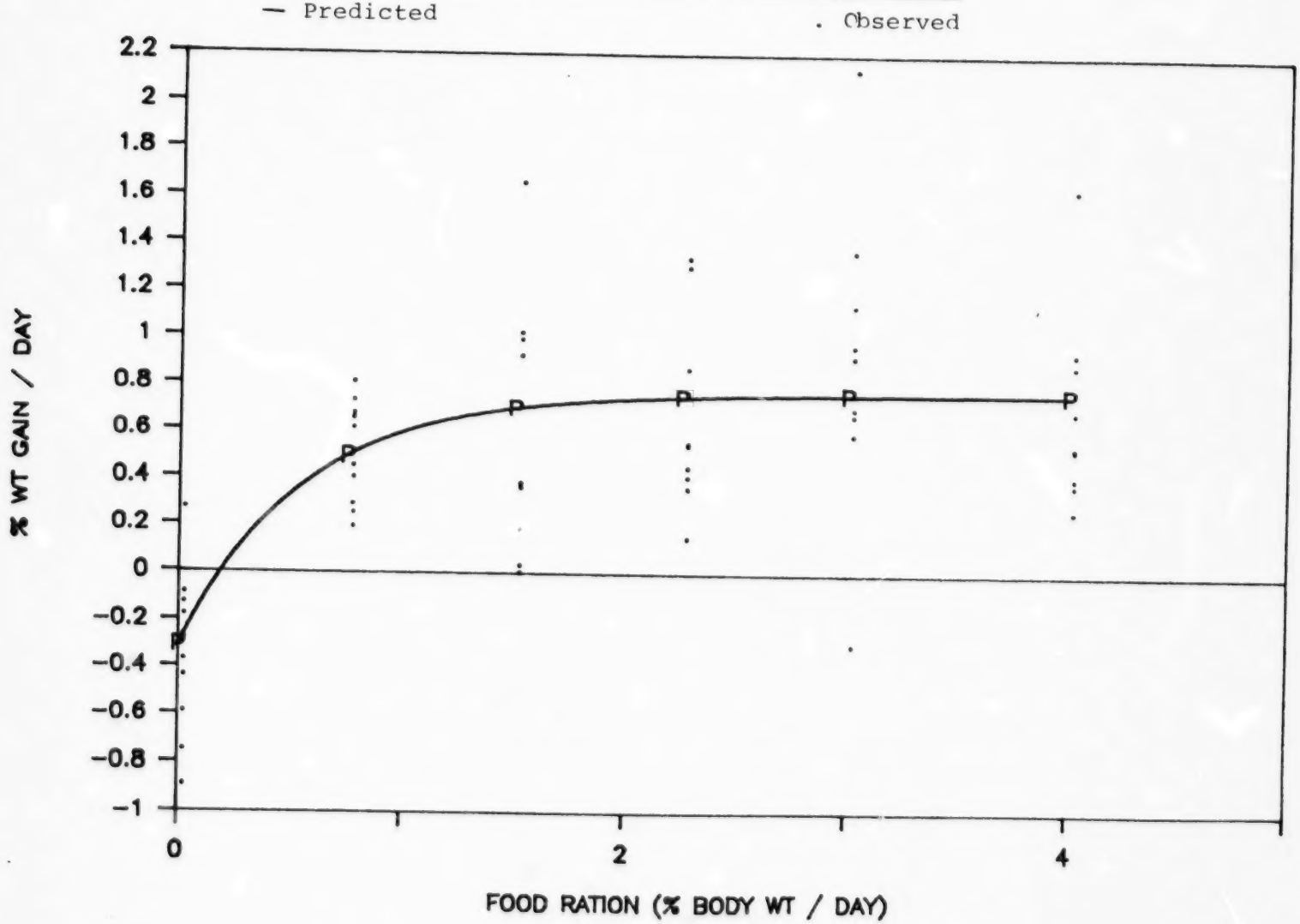


FIGURE 6

Weight Class 15 Grams Normal Probability Plot
of Residuals from Nonlinear Regression

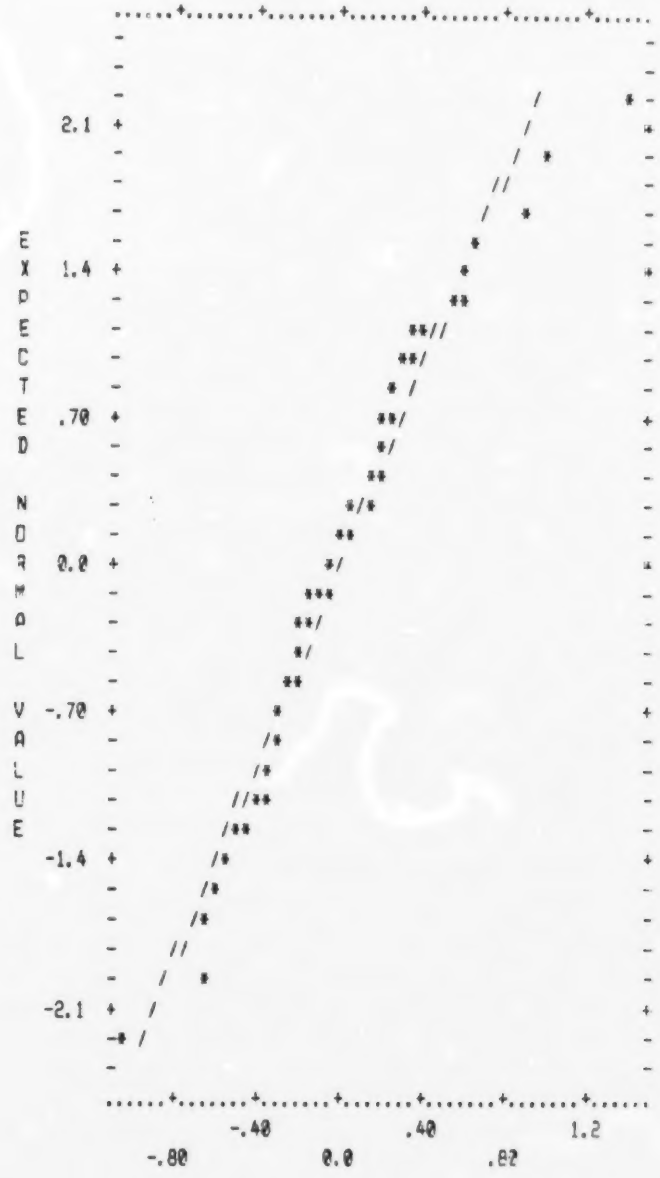
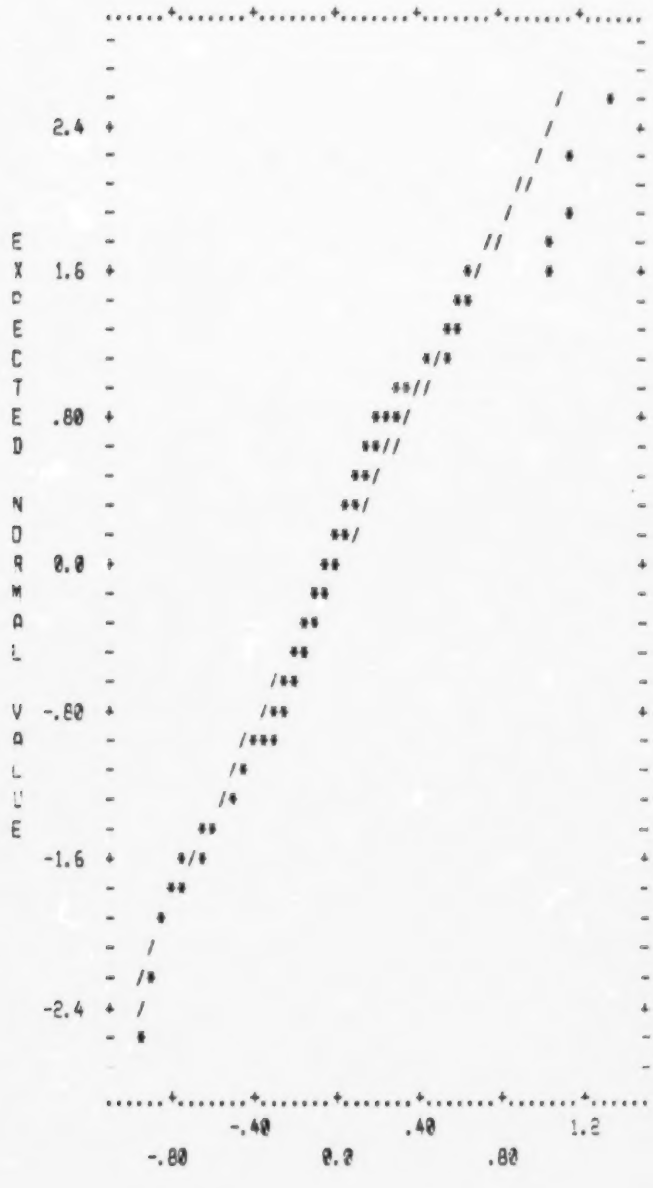


FIGURE 4

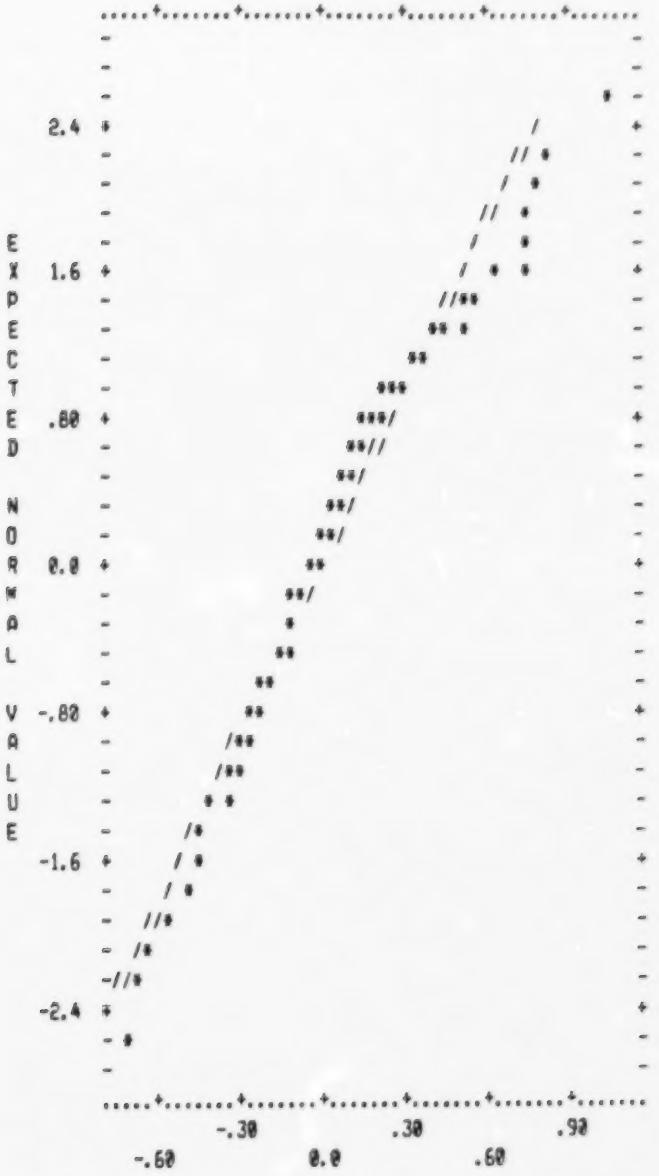
Weight Class 2 Grams Normal Probability Plot
of Residuals from Nonlinear Regression



VALUES FROM NORMAL DISTRIBUTION WOULD LIE
ON THE LINE INDICATED BY THE SYMBOL / .

FIGURE 5

Weight Class 5 Grams Normal Probability Plot
of Residuals from Nonlinear Regression



VALUES FROM NORMAL DISTRIBUTION WOULD LIE
ON THE LINE INDICATED BY THE SYMBOL

FIGURE 7

Weight Class 2 Grams Scatter Plot
of Conversion Efficiency Data with Estimated Conversion Efficiency Curve

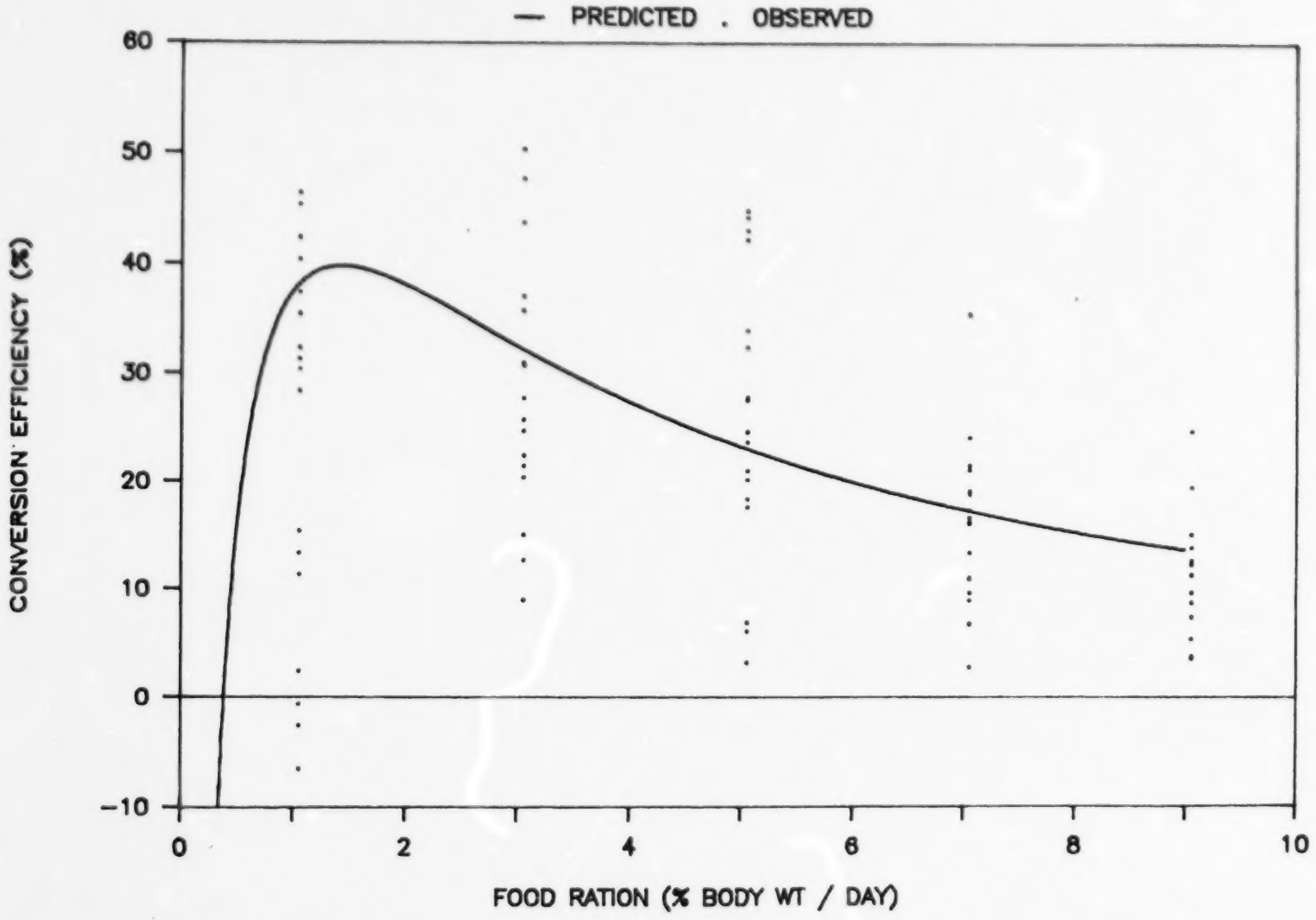


FIGURE 8

Weight Class 5 Grams Scatter Plot
of Conversion Efficiency Data with Estimated Conversion Efficiency Curve

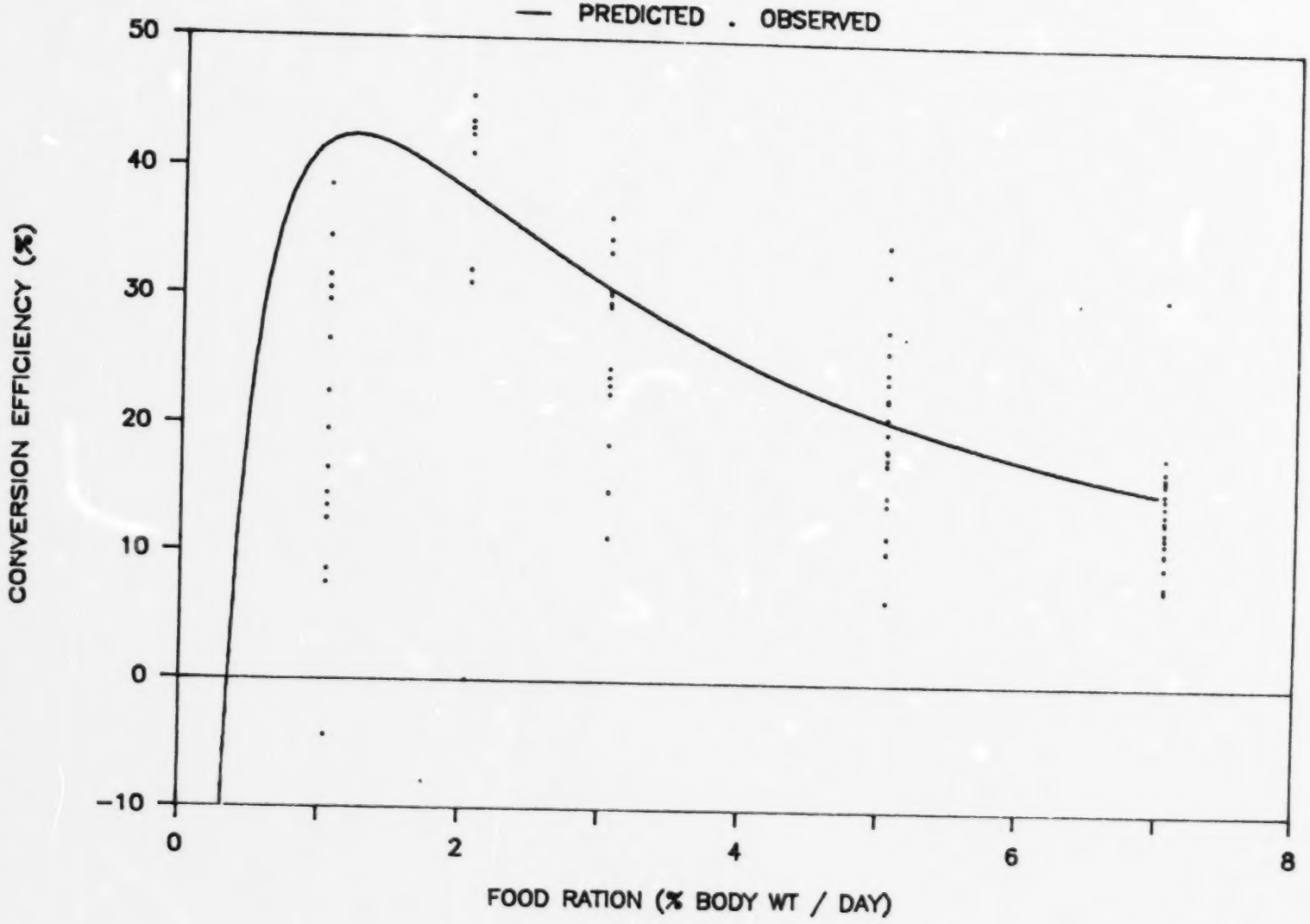
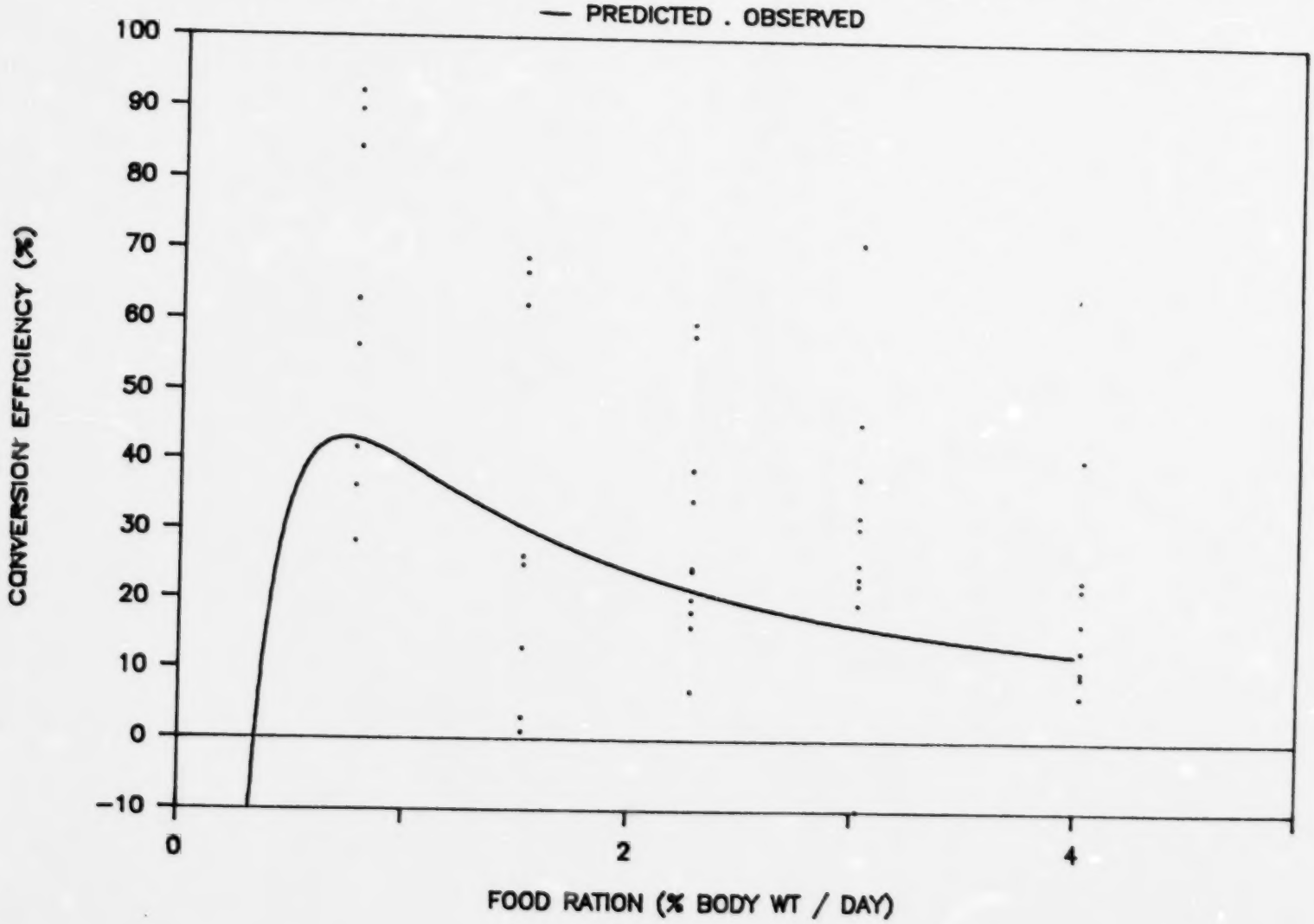


FIGURE 9

Weight Class 15 Grams Scatter Plot of
of Conversion Efficiency Data with Estimated Conversion Efficiency Curve



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